British Journal of Pharmacology (1998) 124, 1261–1269
http://www.stockton-press.co.uk/bjp

Selective inhibition of M-type potassium channels in rat sympathetic neurons by uridine nucleotide preferring receptors

[1]Stefan Boehm

[1]Department of Neuropharmacology, University of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria

1 UTP and UDP depolarize rat superior cervical ganglion neurons and trigger noradrenaline release from these cells. The present study investigated the mechanisms underlying this excitatory action of uridine nucleotides by measuring whole-cell voltage-dependent K^+ and Ca^{2+} currents.

2 Steady-state outward (holding) currents measured in the amphotericin B perforated-patch configuration at a potential of −30 mV were reduced by 10 μM UTP in a reversible manner, but steady-state inward (holding) currents at −70 mV were not affected. This action of UTP was shared by the muscarinic agonist oxotremorine-M. In current-voltage curves between −20 and −100 mV, UTP diminished primarily the outwardly rectifying current components arising at potentials positive to −60 mV.

3 Slow relaxations of muscarinic K^+ currents (I_M) evoked by hyperpolarizations from −30 to −55 mV were also reduced by 10 μM UTP (37% inhibition) and oxotremorine-M (81% inhibition). In contrast, transient K^+-currents, delayed rectifier currents, fast and slow Ca^{2+}-dependent K^+ currents, as well as voltage-dependent Ca^{2+} currents were not altered by UTP.

4 In conventional (open-tip) whole-cell recordings, replacement of GTP in the pipette by GDPβS abolished the UTP-induced inhibition of I_M, whereas replacement by GTPγS rendered it irreversible.

5 The UTP-induced reduction of I_M was half maximal at 1.5 μM with a maximum of 37% inhibition; UDP was equipotent and equieffective, while ADP was less potent (half maximal inhibition at 29 μM). ATP had no effect at $\leqslant$30 μM.

6 The inhibition of I_M induced by 10 μM UTP was antagonized by pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) at $\geqslant$30 μM and by reactive blue 2 at $\geqslant$10 μM, but not by suramin at concentrations up to 30 μM.

7 These results show that rat superior cervical ganglion neurons possess uridine nucleotide preferring P2Y receptors which inhibit K_M channels. This effect presumably forms the basis of the excitatory action of uridine nucleotides in rat sympathetic neurons.

Keywords: UDP; UTP; M currents; rat sympathetic neurons; P2Y receptor; GTP binding protein

Introduction

Receptors for adenine and uridine nucleotides can be divided into two general classes: receptors with intrinsic ion channels (P2X purinoceptors) and receptors coupled to heterotrimeric GTP binding proteins (P2Y purinoceptors; Fredholm *et al*., 1994; North & Barnard, 1997). The G protein-linked nucleotide receptors are heterogeneous as exemplified by diverse agonist specificities and multiple intracellular signal transduction cascades (Harden *et al*., 1995). The identification of DNA and amino acid sequences for at least seven different P2Y purinoceptors corroborates the existence of several subtypes of P2Y receptors (Harden *et al*., 1995; North and Barnard, 1997). At least four types of P2Y purinoceptors are activated by uridine nucleotides: the $P2Y_2$ receptor, previously referred to as $P2_U$ receptor, accepts ATP and UTP as equipotent agonists (Lustig *et al*., 1993; Parr *et al*., 1994); at the $P2Y_4$ receptor, UTP is more potent an agonist than ATP, while UDP is inactive (Nicholas *et al*., 1996); conversely, at mammalian $P2Y_6$ receptors, UDP is more potent than UTP, whereas ATP is inactive (Nicholas *et al*., 1996); the avian $P2Y_3$ receptor shows a similar agonist profile (Webb *et al*., 1996) and may thus represent a species homologue of $P2Y_6$ (Communi & Boeynaems, 1997).

Upon heterologous expression, all these uridine nucleotide-sensitive receptors lead to the activation of phospholipase C with ensuing accumulation of inositol phosphates (North & Barnard, 1997), and heterologously expressed $P2Y_2$ receptors, in particular, inhibit muscarinic (M-type) K^+ (K_M) channels (Filippov *et al*., 1997). Along these lines, UTP-sensitive nucleotide receptors in neuroblastoma glioma hybrid cells have been found to inhibit M-type K^+ channels (Filippov *et al*., 1994) and, in addition, N-type Ca^{2+} channels (Filippov & Brown, 1996). The modulation of voltage-activated Ca^{2+} and K^+ channels also represents a major mechanism by which G protein linked receptors regulate the excitability of neurons (Hille, 1994). Accordingly, P2Y purinoceptors have been found to inhibit neuronal Ca^{2+} channels (Currie & Fox, 1996) and to activate K^+ channels of neurons in primary cell culture (Ikeuchi & Nishizaki, 1996), but in these cases UTP was either less active than ATP or even inactive.

Rat superior cervical ganglion neurons possess at least two different types of nucleotide receptors which are both excitatory (Connolly *et al*., 1993) and trigger noradrenaline release (Boehm, 1994; Boehm *et al*., 1995). One of these receptors is a ligand-gated ion channel preferentially activated by adenine nucleotides, i.e. a P2X purinoceptor. The other receptor recognizes uridine nucleotides at low micromolar concentrations as agonists and appears to be metabotropic rather than ionotropic (Boehm *et al*., 1995; Boehm & Huck, 1997a). Recently, these results have been corroborated in neurons from thoracolumbal paravertebral sympathetic ganglia of the rat, but the signalling mechanisms of the uridine

[1] Author for correspondence.

nucleotide receptor remained elusive (von Kügelgen *et al.*, 1997). Experiments measuring [^{3}H]noradrenaline release from superior cervical ganglion neurons *in vitro* indicated that an inhibition of K_M channels through activation of neurotransmitter receptors may be sufficient to trigger action potential-mediated transmitter release (Boehm & Huck, 1997b). Results obtained in bullfrog sympathetic neurons indicated that uridine nucleotides at concentrations of about 100 μM can reduce M currents (I_M; Adams *et al.*, 1982). Therefore, the present study investigated the actions of uridine and adenine nucleotides on voltage-dependent Ca^{2+} and K^+ currents in neurons from rat superior cervical ganglia and focused specifically on I_M. The results show that G protein-coupled receptors preferentially activated by uridine nucleotides inhibit K_M, but no other type of K^+ channels nor voltage-activated Ca^{2+} channels.

Methods

Cell culture

Superior cervical ganglia were dissected from 2–6 day-old Sprague Dawley rat pups and dissociated enzymatically as described previously (Boehm, 1994). Cells were resuspended in Dulbeccos modified Eagle's Medium (Gibco #041-01885M) containing 2.2 g/l glucose, 10 mg/l insulin, 25000 IU/l penicillin and 25 mg/l streptomycin (Gibco #043-05140D), 10 μg/l nerve growth factor (Gibco #0436050), and 5% fetal calf serum (Gibco #011-0620H) and plated onto 35 mm culture dishes (Nunc #153066) coated with poly-D-lysine (Sigma, 25 mg/l). Secretagogue effects of uridine nucleotides have been detected in rat superior cervical ganglion neurons kept in primary cell culture for 7 days (Boehm *et al.*, 1995). Therefore, the present electrophysiological experiments were also performed after 6–7 days *in vitro*.

Electrophysiology

Membrane currents were recorded at room temperature (20–24°C) from the somata of isolated neurons in either the amphotericin B perforated-patch (Rae *et al.*, 1991) or the conventional open-tip whole-cell (Hamill *et al.*, 1981) configuration of the patch-clamp technique using a List EPC-7 amplifier (List Medical, Darmstadt, Germany) and the Pclamp 6.0 hard- and software (Axon Instruments, Foster City, CA, U.S.A.). Experiments focused on the following K^+ currents: (i) muscarinic (M-type) K^+ currents (I_M) were activated at −30 mV and quantified by determining amplitudes of the slow current deactivation relaxations during 1 s hyperpolarizing voltage steps to −55 mV (see Figure 2a; Constanti & Brown, 1981; Adams *et al.*, 1982); (ii) transient, voltage-dependent K^+ currents (I_A) were elicited by 100 ms depolarizations from −100 to +10 mV; (iii) delayed rectifier K^+ currents (I_{DR}) were evoked by 100 ms depolarizations to +10 mV from a holding potential of −50 mV at which I_A of rat sympathetic neurons is completely inactivated (Schofield & Ikeda, 1989); (iv) fast, Ca^{2+}-activated K^+ currents (I_C) were induced by 30 ms voltage steps from −50 to +10 mV (Marsh & Brown, 1991); (v) slow, Ca^{2+}-activated K^+ currents which mediate after hyperpolarization (I_{AHP}) were measured at −50 mV subsequently to 50 ms depolarizations to +30 mV (Pennefather *et al.*, 1985). In addition, voltage-activated Ca^{2+} current were evoked by 30 ms depolarizations from −80 to 0 mV (Boehm & Huck, 1995).

The extracellular (bathing) solution contained (mM) NaCl (140), KCl (6.0), $CaCl_2$ (2.0), $MgCl_2$ (2.0), glucose (20), HEPES (10), adjusted to pH 7.4 with NaOH. Tetrodotoxin (TTX; 0.3 to 1 μM) was included to suppress voltage-activated Na^+ currents, unless indicated otherwise. 100 μM Cd^{2+} was added to reduce Ca^{2+} entry via voltage-gated Ca^{2+} channels in order to minimize the contribution of Ca^{2+}-dependent K^+ channels when I_A and I_{DR} were investigated. For the investigation of the Ca^{2+}-dependent K^+ currents, I_C and I_{AHP}, 1 mM 4-aminopyridine (4-AP) was added to the bathing solution to suppress I_{DR} (Marsh & Brown, 1991). All channel blocking agents and neurotransmitter receptor agonists were applied via a DAD-12 drug application device (Adams & List) which permits a complete exchange of solutions surrounding the cells under investigation within less than 100 ms (see e.g. Boehm & Betz, 1997).

Whole-cell I_M and I_{AHP} were recorded by the amphotericin B perforated-patch technique (Rae *et al.*, 1991) to prevent rundown which is prominent under open-tip whole-cell recording conditions (compare Figure 4 and 5a). For this purpose, patch pipettes pulled (Flaming-Brown puller, Sutter Instruments) from borosilicate glass capillaries (Science Products, Frankfurt/Main, Germany) were first front-filled with a solution consisting of (mM) K_2SO_4 (75), KCl (55), $MgCl_2$ (8), and HEPES (10), adjusted to pH 7.3 with KOH. Then, electrodes were back-filled with the same solution containing 200 μg ml^{-1} amphotericin (in 0.8% DMSO) which yielded tip resistencies of 1 to 3 MΩ. In order to apply GTP or related nucleotides to the cytosol of the neurons under investigation, I_M was recorded in the conventional (open-tip) whole-cell configuration with an intracellular (pipette) solution containing (mM) K-aspartate (120), KCl (30), $CaCl_2$ (3.18), EGTA (5), HEPES (10), Mg-ATP (2), and Li-GTP (2), adjusted to pH 6.8 with NaOH. This solution was also used to measure I_A, I_{DR}, and I_C in open-tip whole-cell recordings. Where indicated, 2 mM GTP was replaced by either 2 mM GDPβS or by 0.2 mM GTPγS. Voltage-activated Ca^{2+} currents were also measured in the open-tip whole-cell configuration, as these currents showed distorted kinetics when determined in the perforated-patch mode (not shown). The intracellular solution for Ca^{2+} current recordings contained (mM) CsCl (120), tetraethylammonium chloride (20), $CaCl_2$ (0.24), glucose (10), HEPES (10), EGTA (5), Mg-ATP (2), and Li-GTP (2), adjusted to pH 7.3 with KOH.

Calculations

I_M was quantified by measuring amplitudes of slow M current deactivation relaxations during 1 s hyperpolarizing voltage steps from −30 to −55 mV (Constanti & Brown, 1981; Marrion *et al.*, 1989); the difference between current amplitudes 20 ms after the onset of hyperpolarizations and 20 ms prior to re-depolarization was taken as a measure for I_M. I_A was determined as the peak current amplitude during 100 ms voltage steps from −100 to +10 mV. Current amplitudes at the end of 100 ms depolarizations from −50 mV to +10 mV were taken as a measure for I_{DR}. For the quantification of I_C, current amplitudes were averaged from 5 to 10 ms after the beginning of a 30 ms depolarization from −50 to +10 mV; I_{DR} (which was blocked by 4-aminopyridine) activates more slowly than I_C and therefore may contribute to the current at this period of time only to a minor extent (see Figure 2d). I_{AHP} was recorded at −50 mV and quantified by averaging current amplitudes from 20 to 40 ms after repolarization from +30 mV. Ca^{2+} currents were quantified

by measuring peak amplitudes during the 30 ms depolarization to 0 mV.

All types of currents were evoked once every 20 s, and amplitudes obtained during the application of test drugs for periods of 2 min (b) were compared to those measured before (a) and after (c) application of these drugs by calculating 200b/(a+c)=% of control or 100-(200b/[a+c])=% inhibition. To evaluate the effects of nucleotide receptor antagonists (suramin, PPADS, reactive blue 2), neurons were first exposed to these agents for 2 min, and then UTP was applied together with antagonist.

All data represent arithmetic means±s.e.mean; n represent numbers of single neurons. Concentration-response curves were fitted to experimentally obtained data by the ALLFIT programme (DeLean *et al*., 1978) which provides estimates (±s.e.m.) for half maximal concentrations as well as maximal effects and determines the qualities of fitted results. Significance of differences between single data points was evaluated by the Mann–Whitney test.

Materials

Na-UTP, Na-UDP, Na_2-ATP, Na-ADP, Li-GTP, Li_3-GDPβS, Li_4-GTPγS, reactive blue 2 (basilene blue E3G), hexokinase type III (# H-5000), and amphotericin B were purchased from Sigma (Deisenhofen, Germany); Oxotremorine M (Oxo-M), pirenzepine dihydrochloride, D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin (Hoe 140), 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (U.K. 14,304), pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium (PPADS), and linopirdine from RBI (Natick, MA, U.S.A.); tetrodotoxin from Latoxan (Rosans, France). Suramin was a gift of Bayer, Austria.

Results

Voltage-dependent effects of UTP on steady membrane currents of rat superior cervical ganglion neurons

When sympathetic neurons were voltage-clamped at potentials positive to −60 mV, they displayed steady-state outward holding currents. At −30 mV, for instance, these outward currents had amplitudes of 383.2±38.7 pA (n=21). Superfusion of the neurons with 10 μM UTP caused a reduction of these outward currents measured at −30 mV which followed a biexponential time course. At more hyperpolarized potentials, such as −70 mV, small inward, instead of outward, currents were observed, and UTP had no effect on these currents (Figure 1a). Outward currents at slightly depolarized potentials are carried by muscarine-sensitive (M-type) K^+ channels, which can be blocked by the activation of M_1 muscarinic receptors (Marrion *et al*., 1989; Bernheim *et al*., 1992). In the present experiments, the muscarinic agonist Oxo-M (10 μM), like UTP, caused a reduction of outward currents at −30 mV, but not of inward currents at −70 mV (Figure 1a). The reduction of outward currents in the presence of UTP developed more slowly than that caused by Oxo-M. Likewise, after removal of the agonists, currents returned towards the baseline more slowly when UTP had been applied than when Oxo-M had been applied (Figure 1a).

To corroborate that UTP had effects only at depolarized membrane potentials, current-voltage curves were generated by application of slow ramp hyperpolarizations from −20 to −100 mV (Figure 1B). Current-voltage (I-V) relations showed outward rectification at potentials positive to −60 mV, as described previously (Jones *et al*., 1995; Lamas *et al*., 1997). UTP (10 μM) affected primarily this outwardly rectifying current component at the I-V curve (Figure 1b). Hence, UTP exerted its action at membrane potentials at which I_M is activated (Constanti & Brown, 1981).

UTP selectively modulates I_M

To investigate the effect of UTP on I_M in more detail, 25 mV hyperpolarizations from a holding potential of −30 mV were applied every 20 s. Subsequent to instantaneous current steps, these hyperpolarizations caused slow current relaxations with amplitudes of 136.1±7.2 pA (n=21). The current decayed in an apparently biexponential manner with a predominant (62.2±6.7% of the current) fast time constant of 49.3±2.6 ms and a minor (37.8±3.9%) slow time constant of 387.8±53.7 ms (n=12). This agrees well with the kinetic parameters previously reported for I_M relaxations in this preparation (Marrion *et al*., 1989; Lamas *et al*., 1997). Furthermore, these slow current relaxations were reduced by

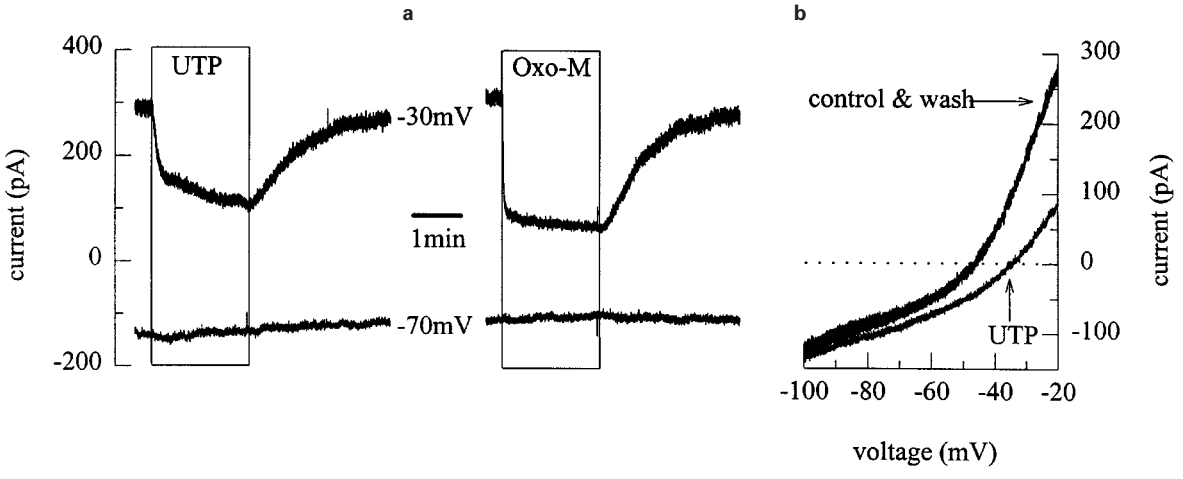

Figure 1 Voltage-dependence of the effect of UTP on steady membrane currents of rat superior cervical ganglion neurons measured in the perforated-patch configuration. (a) Effects of 10 μM UPT and 10 μM Oxo-M on steady-state membrane currents recorded at −30 mV and −70 mV, respectively. (b) Effect of UTP on currents elicited by slow ramp hyperpolarizations from −20 to −100 mV at a rate of about 8 mV s^{-1}. Traces were obtained before (control) and at the end of a 2 min application of 10 μM UTP, as well as subsequent to a 3 min washout period (wash). Recordings in a and b were obtained in different neurons.

48.2±3.1% in the presence of 1 mM Ba^{2+}, and by 60.0±1.7% in the presence of 10 μM linopirdine ($n=5$). At these concentrations, the two agents selectively block K_M channels (Adams *et al.*, 1982; Lamas *et al.*, 1997). Hence, these current relaxations most likely reflect the closure of K_M channels.

Application of 10 μM UTP for periods of 2 min reduced I_M relaxation amplitudes by 37% (Figure 2a and Table 1). However, the inhibitory effect of UTP was highly variable between different neurons (even within neurons of one culture dish), and the reduction of I_M relaxations ranged from 9.5 to 97.6%. Nevertheless, the values of I_M reduction showed a Gaussian distribution, as indicated by a Kolmogorov-Smirnov test ($P>0.1$). For comparison, Oxo-M (10 μM) reduced I_M relaxation amplitudes by 81.2±1.2% ($n=5$), and the inhibition ranged from 76.9% to 84.1%. The kinetics of I_M relaxations were not altered by UTP, the time constants being 45.2±2.0 ms and 455.7±93.4 ms ($P>0.1$ vs control; $n=12$). This is again in accordance with the finding that Oxo-M also reduces I_M relaxations without altering their time course

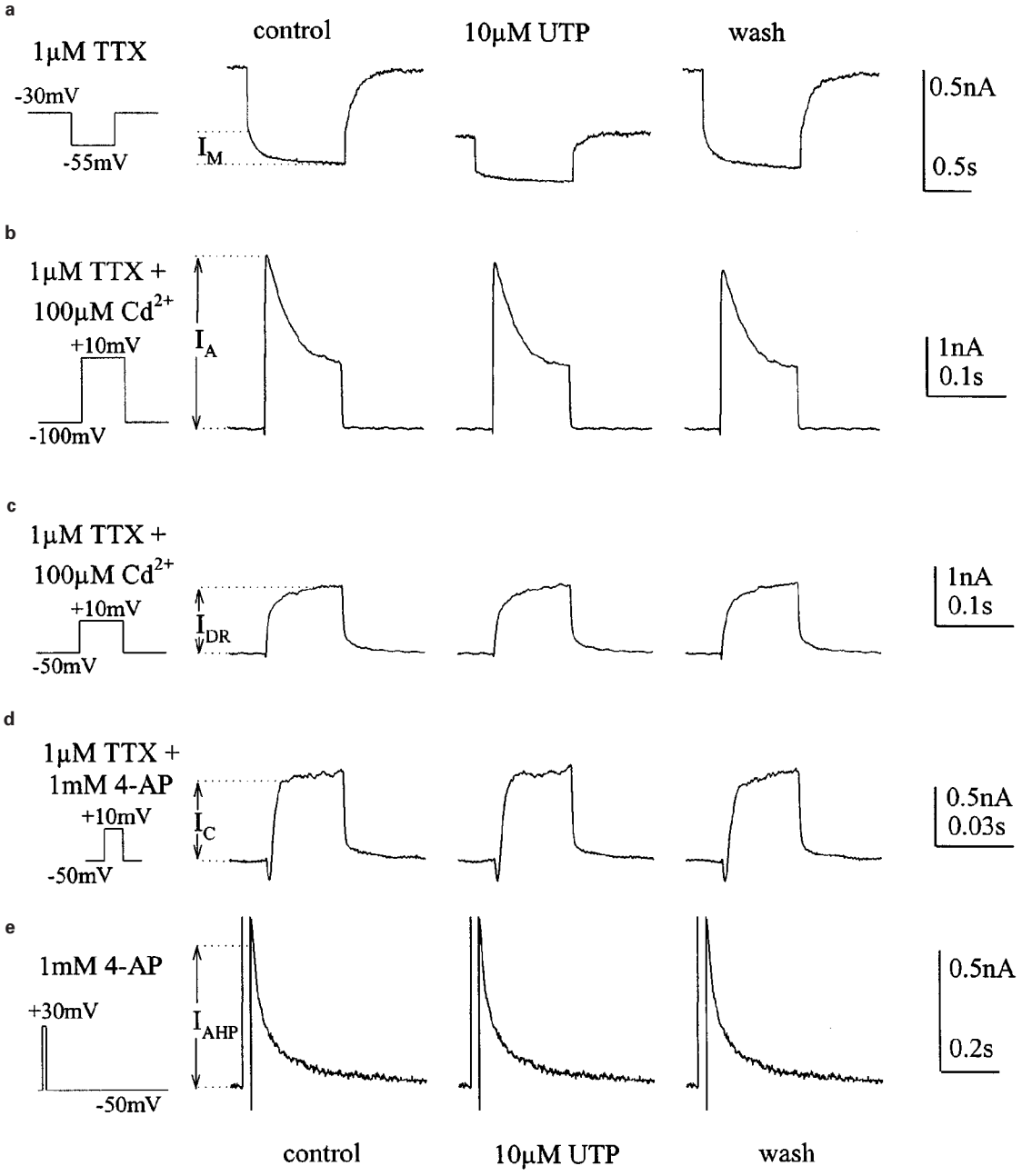

Figure 2 Effects of UTP on I_M, I_A, I_{DR}, I_C and I_{AHP} in rat superior cervical ganglion neurons. Currents were evoked every 20 s by the pulse protocols indicated. The traces shown were recorded before (control), at the end of a 2 min application of 10 μM UTP, and subsequent to a 3 min washout period (wash). (a) I_M relaxations were induced by 1 s hyperpolarizations from −30 to −55 mV in the perforated-patch configuration in the continuous presence of 1 μM TTX. (b) I_A was elicited by depolarizations from −100 to +10 mV in the open-tip whole-cell configuration in the continuous presence of 1 μM TTX and 100 μM $CdCl_2$. (c) I_{DR} was evoked by depolarizations from −50 to +10 mV in the open-tip whole-cell configuration in the continuous presence of 1 μM TTX and 100 μM $CdCl_2$. Currents in b and c were obtained in the same neuron. (d) I_C, like I_{DR}, was elicited by depolarizations from −50 to +10 mV in the open-tip whole-cell configuration in the continuous presence of 1 μM TTX and 1 mM 4-aminopyridine (4-AP). (e) I_{AHP} was determined subsequently to 50 ms depolarizations to +30 mV at a potential of −50 mV in the perforated-patch configuration in the continuous presence of 1 mM 4-aminopyridine (4-AP).

(Marrion *et al*., 1989). To find out whether the action of UTP was specific for I_M, the following other K^+ currents were also recorded: Transient A currents (I_A) were determined by measuring peak outward current amplitudes due to depolarizations from −100 to +10 mV (Figure 2b). Delayed rectifier currents (I_{DR}) hardly contribute to these peak currents as they are activated more slowly; these currents were isolated by changing the holding potential to −50 mV (Figure 2c) which entirely inactivates I_A (Schofield & Ikeda, 1989). These two types of K^+ currents were measured in the presence of 100 μM Cd^{2+} to reduce Ca^{2+}-dependent K^+ currents. Conversely, fast (I_C; Figure 2d) and slow (I_{AHP}; Figure 2e) Ca^{2+}-dependent K^+ currents were determined in the absence of Cd^{2+}, but in the presence of 1 mM 4-AP which reduces I_{DR} by about 75% (Marsh & Brown, 1991). None of these K^+ conductances were affected by UTP (Figure 2 and Table 1).

In addition to K^+ currents, voltage-activated Ca^{2+} currents were measured, and the effect of UTP was investigated. Ca^{2+} currents evoked by depolarizations from −80 to 0 mV were not altered in the presence of 10 μM UTP (100.1±2.3% of control; $n=5$), but clearly reduced when the α_2-adrenergic agonist UK 14,304 (10 μM; 58.2±8.6% of control; $n=5$) or Oxo-M (10 μM; 50.8±10.2% of control; $n=5$) were applied (Figure 3).

Table 1 Different K^+ currents (I_M, I_A, I_{DR}, I_C, I_{AHP}) in rat superior cervical ganglion neurons and their modulation by 10 μM UTP

Current	n	*Amplitude (pA)*	*Alteration by UTP (% of control)*
I_M	25	115.4±10.5	63.2±4.1
I_A	6	2366.4±373.8	101.7±3.6
I_{DR}	6	1281.9±123.8	99.9±1.7
I_C	6	575.8±49.2	104.6±4.7
I_{AHP}	7	347.3±57.5	99.6±1.5

Currents were elicited by the pulse protocols shown in Figure 2 and were recorded in either the open-tip (I_A, I_{DR}, I_C) or the amphotericin perforated-patch (I_M, I_{AHP}) whole-cell configuration. 10 μM UTP was applied for periods of 2 min as shown in Figure 4a.

The UTP-induced inhibition of I_M is altered by intracellular hydrolysis-resistant guanine nucleotides

UTP- and UDP-sensitive nucleotide receptors belong to the superfamily of G protein-linked receptors (Fredholm *et al*., 1994; North & Barnard, 1997). Therefore, the inhibition of I_M by UTP was investigated in the open-tip whole-cell configuration, with GTP (2 mM), GDPβS (2 mM), or GTPγS (0.2 mM) added to the intracellular solution. Steady-state outward currents measured at −30 mV as well as I_M relaxations showed considerable rundown under these recording conditions (Figure 4). Nevertheless, UTP (10 μM) caused at least partially reversible reductions of outward currents at −30 mV and of I_M relaxations when the pipette solution contained GTP (Figure 4a): the inhibition of I_M relaxations by UTP amounted

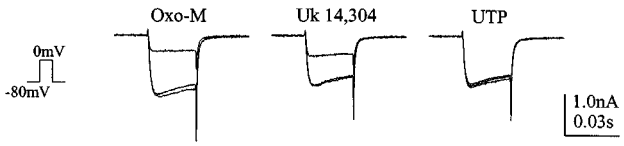

Figure 3 Voltage-activated Ca^{2+} currents are reduced by an α_2-adrenergic agonist and by Oxo-M, but not by UTP. Ca^{2+} currents were evoked by depolarizations from −80 to 0 mV in one superior cervical ganglion neuron. The traces show recordings obtained before, during and after application of Oxo-M, the α_2-adrenergic agonist UK 14,304, and UTP (each at 10 μM), respectively.

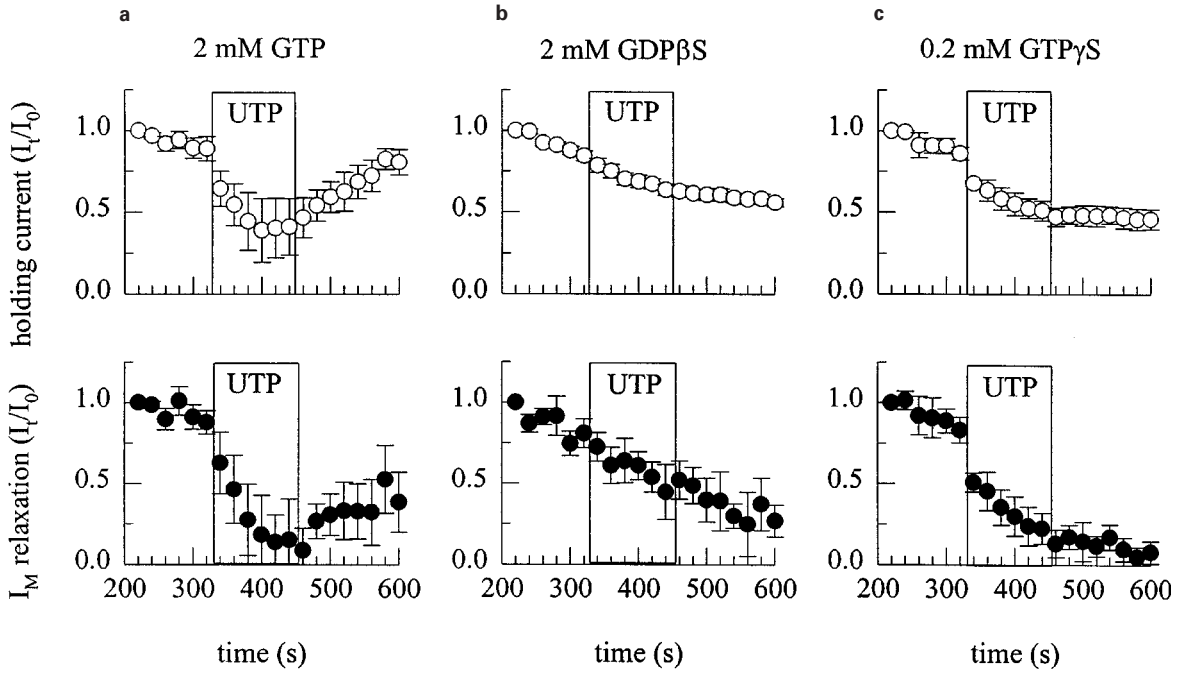

Figure 4 Effects of intracellular GTP analogues on the UTP-induced inhibition of I_M recorded in the open-tip whole-cell configuration. I_M relaxations were evoked by 1 s hyperpolarizations from −30 to −55 mV as shown in Figure 2a. The upper graphs (white circles) show normalized holding current amplitudes, whereas the lower graphs (black circles) depict normalized amplitudes of I_M relaxations. Recordings were started approximately 3 min after formation of the whole-cell configuration. In (a) the intracellular solution contained 2 mM GTP, which was replaced by 2 mM GDPβS in (b) and by 0.2 mM GTPγS in (c). $n=4$ in each graph.

to 44.8±11.4% ($n=4$). Replacement of GTP by GDPβS which traps heterotrimeric GTP binding proteins in an inactive conformation abolished the UTP-induced inhibition (1.9±1.8% inhibition; $n=4$; $P<0.05$ vs GTP) of I_M relaxations (Figure 4b). Replacement of GTP by GTPγS which traps the G proteins in an active conformation accelerated the inhibition by UTP and rendered the effect irreversible (Figure 4c). The amplitudes of I_M relaxations measured prior to the application of UTP were not significantly affected by exchanging intracellular guanosine nucleotides (not shown).

I_M is inhibited by UTP, UDP, ADP, but not by ATP

In an initial set of five neurons, the action of 10 μM UTP was compared to that of UDP and ADP, both at 10 μM: UTP (40.0±8.2% inhibition) and UDP (37.5±6.9% inhibition) reduced I_M relaxations to the same extent, whereas ADP (11.8±2.5% inhibition; $P<0.05$) was significantly less active. At 300 μM, however, the inhibition by ADP (27.6±4.7% inhibition; $P>0.05$) was not significantly different from the action of UTP (37.9±8.3% inhibition) and UDP (35.4±7.3% inhibition).

In subsequent experiments, the action of UTP on I_M turned out to be concentration-dependent in two ways: first, the speed of I_M reduction increased when concentrations were raised from 1 to 100 μM (Figure 5a); second, the magnitude of I_M reduction increased in a concentration-dependent manner, the effect being half maximal at 1.5±0.4 μM and reaching a maximum of 37.0±4.8% inhibition (Figure 5b). UDP also reduced the amplitudes of steady-state outward currents at −30 mV as well as I_M relaxations in a manner identical to that of UTP (Figure 5a). To account for differences in the inhibitory action of nucleotides between different cells, actions of ADP, ATP, and UDP were compared to the effect of 10 μM UTP in the very same cell. Concentration-response curves for the reduction of I_M relaxations obtained in this manner revealed that the action of UDP was half maximal at 1.3±0.3 μM and reached the same maximum as UTP (Figure 5b). ATP, at 10 and 30 μM, failed to cause any alteration in I_M, but ADP was a weak agonist with half maximal effects at 28.8±10.4 μM (Figure 5b).

Conversion of UDP to UTP as well as UTP contaminations in commercially available UDP preparations may cause problems in the interpretation of concentration-response relations obtained with UDP (Nicholas *et al*., 1996). For this reason, UDP (1 mM) was first treated with hexokinase (10 U ml^{-1} for 1 h in the presence of 20 mM glucose) and then applied in the continuous presence of 1 U ml^{-1} hexokinase to prevent conversion to UTP (Nicholas *et al*., 1996). However, 10 μM hexokinase-treated UDP reduced I_M relaxations to the same extent (31.6±2.7% inhibition; $n=5$) as 10 μM untreated UDP (28.2±5.4% inhibition; $n=5$; $P>0.1$) and 10 μM UTP (27.3±6.4% inhibition; $n=5$; $P>0.1$).

The UTP-induced inhibition of I_M is antagonized by reactive blue 2 and PPADS, but not by suramin

Reactive blue 2, PPADS, and suramin, antagonists at several types of P2 purinoceptors, have been used to discriminate between various nucleotide receptors (Charlton *et al*., 1996; Communi *et al*., 1996a; Robaye *et al*., 1997). In the present experiments, 10 or 30 μM suramin did not alter the inhibition of I_M relaxations by 10 μM UTP. PPADS significantly reduced the inhibitory action of UTP (10 μM) at a concentration of 30 μM, but had no antagonistic effect at 10 μM. Reactive blue 2 largely attenuated the inhibition by 10 μM UTP at both concentrations used, 10 or 30 μM (Figure 6). Antagonistic effects of both, PPADS and reactive blue 2, were reversible within 2 min (not shown). In the presence of each of three P2 purinoceptor antagonists, amplitudes of I_M relaxations (in the absence of UTP) were increased, but these changes were not statistically significant (not shown). Pirenzepine (10 μM) and Hoe 140 (100 nM) which antagonize the inhibition of I_M caused by the activation of muscarinic (Marrion *et al*., 1989) and bradykinin (Jones *et al*., 1995) receptors, respectively, did not alter the inhibitory action of UTP (UTP: 30.5±2.6% inhibition; UTP+pirenzepine: 33±2.6% inhibition; UTP+Hoe 140: 28.9+3.7% inhibition; $n=4$; $P>0.1$).

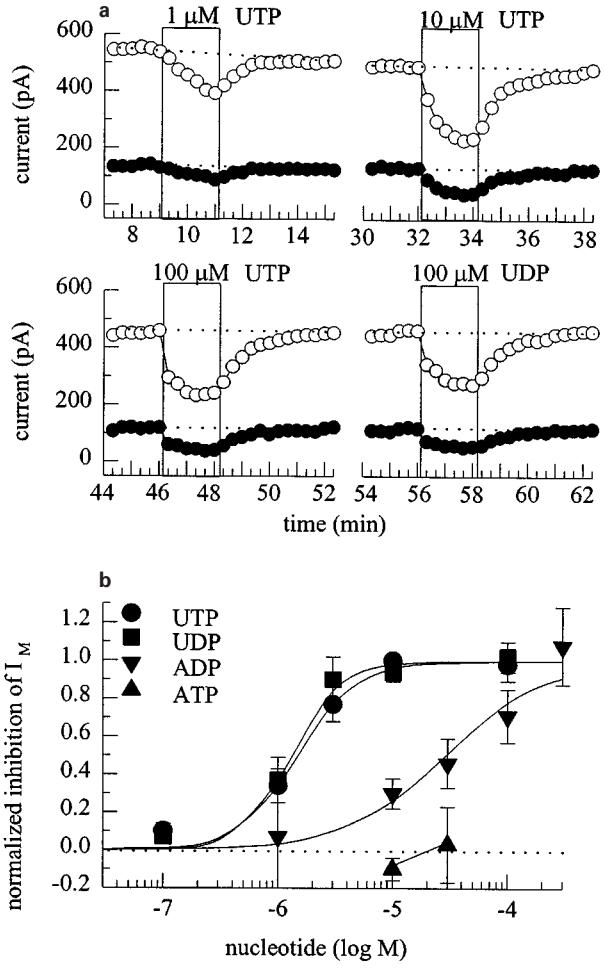

Figure 5 Modulation of I_M recorded in the perforated-patch configuration by uridine and adenine nucleotides. (a) Time course of the inhibition of I_M by 1, 10, and 100 μM UTP as well as by 100 μM UDP in one rat superior cervical ganglion neuron: at a holding potential of −30 mV, 1 s hyperpolarizations to −55 mV were applied every 20 s (as shown in 2a), and the graphs depict amplitudes of holding currents at −30 mV (white circles) and of I_M relaxations (black circles). (b) Concentration-response curves for the reduction of I_M relaxations by UTP, UDP, ADP, and ATP; I_M relaxations were elicited as shown in Figure 2a, and the indicated concentrations of nucleotides were applied for periods of 2 min as shown in a. To account for differences in the amount of inhibition between single cells, the reduction of I_M relaxation by each nucleotide concentration was normalized to the reduction caused by 10 μM UTP in the very same neuron. The inhibition by 10 μM UTP amounted to 37.0±4.8% ($n=14$). For all other nucleotide concentrations, $n=5$ to 7.

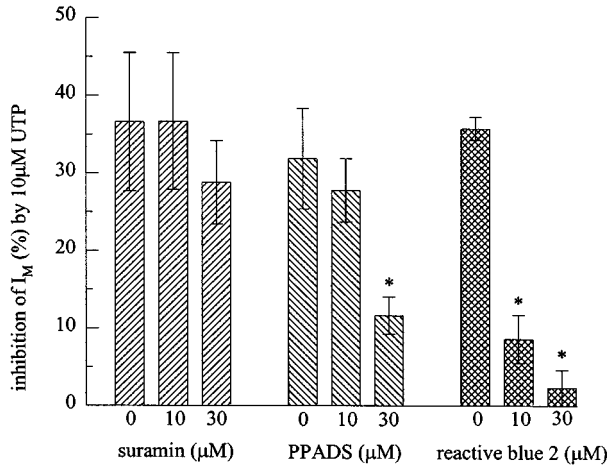

Figure 6 Effects of suramin, PPADS, and reactive blue 2 on the inhibition of I_M by UTP. The bars indicate the reduction of I_M relaxations by 10 μM UTP in the absence and presence of the indicated concentrations of suramin, PPADS, and reactive blue 2, respectively. I_M relaxations were elicited as shown in Figure 2a, and UTP was applied alone or together with antagonists for periods of 2 min. $n=4$ for each antagonist; $^*P<0.05$ vs the action of UTP in the absence of antagonists.

Discussion

Closure of M-type K^+ channels is the major mechanism by which activation of muscarinic acetylcholine receptors (Constanti & Brown, 1981) and of bradykinin receptors (Jones *et al*., 1995) depolarizes rat superior cervical ganglion neurons and increases their excitability. In addition, agonists at these receptors induce Ca^{2+}-dependent and action potential-mediated [^{3}H]noradrenaline release from the very same neurons in cell culture (Boehm & Huck, 1997b, and unpublished observations). Likewise, UTP and UDP cause depolarization of rat superior cervical ganglion neurons (Connolly *et al*., 1993), on one hand, and trigger transmitter release from these neurons (Boehm *et al*., 1995), on the other hand. The present results show that G protein-coupled receptors for uridine nucleotides, similarly to muscarinic and bradykinin receptors, inhibit M-type K^+ channels.

Most commonly, activation of neurotransmitter receptors that inhibit K_M channels also affects other K^+ conductances which may contribute to the excitatory action of these receptors (Jones & Adams, 1987). However, in the present study no evidence could be obtained for a modulatory action of UTP on any type of K^+ current other than I_M. Under conditions which favour the isolation of I_A, I_{DR}, I_C, and I_{AHP}, UTP did not affect outward currents. By contrast, the following findings indicate that UTP did inhibit I_M: (i) Steady outward currents at potentials positive to -60 mV, which are carried primarily by M type channels (Constanti & Brown, 1981), were slowly reduced by UTP and by the muscarinic agonist, Oxo-M, which was used as a control; (ii) slow current relaxations due to hyperpolarizations from -30 to -55 mV were reduced by UTP in a concentration-dependent manner and by Oxo-M, as shown before (Constanti & Brown, 1981); these current relaxations were also reduced by Ba^{2+} and linopirdine which at the concentrations used selectively inhibit K_M channels (Adams *et al*., 1982; Lamas *et al*., 1997); (iii) in current-voltage curves between -100 and -20 mV, primarily outwardly rectifying currents at potentials positive to -60 mV, which arise due to the opening of M type channels (Jones *et al*., 1995; Lamas *et al*., 1997), were affected by UTP. Taken together, UTP acted selectively upon I_M.

In addition to voltage-dependent K^+ channels, G protein-coupled receptors of sympathetic neurons frequently modulate voltage-activated Ca^{2+} channels (Hille, 1994). Accordingly, the α_2-adrenergic agonist UK 14,304 and the muscarinic agonist Oxo-M both caused significant reductions in the amplitudes of voltage-dependent Ca^{2+} currents. However, UTP failed to exert a similar action, which corroborates a recent report showing that Ca^{2+} currents in rat superior cervical ganglion neurons are not affected by UTP, unless the neurons had been injected with $P2Y_2$ purinoceptor cRNA (Filippov *et al*., 1997). Muscarinic receptors are believed to use a common diffusible second messenger to inhibit L-type Ca^{2+} as well as M-type K^+ channels (e.g. Hille *et al*., 1994). Hence, it is somewhat unexpected that UTP failed to modulate voltage-activated Ca^{2+} currents, and one might argue that a soluble second messenger could have been lost during the open-tip whole-cell measurement of Ca^{2+} currents. However, the UTP-induced reduction of I_M was clearly detectable in open-tip whole-cell recordings (see Figure 4) which indicates that the signalling cascade of UTP-sensitive receptors is not entirely lost during this experimental procedure.

Muscarinic acetylcholine and bradykinin receptors inhibit I_M in rat sympathetic neurons via heterotrimeric GTP binding proteins of the Gq family (Caulfield *et al*., 1994; Jones *et al*., 1995). However, these receptors were not involved in the action of UTP as the specific antagonists pirenzepine and Hoe 140 did not alter the UTP-induced inhibition of I_M. Nevertheless, the effect of UTP was mediated by GTP binding proteins, as indicated by the following results: (i) in conventional (open-tip) whole-cell recordings, replacement of intracellular GTP by GDPβS abolished the inhibitory action of UTP on steady-state outward currents at -30 mV and on I_M relaxations; (ii) replacement by GTPγS, in contrast, accelerated the inhibitory action of UTP and rendered it irreversible. Previously, analogous data had been obtained with the inhibition of I_M by muscarinic agonists (Brown *et al*., 1989), results that provided first evidence for a role of G proteins in the muscarinic modulation of M-type K^+ channels. As nucleotide receptors activated by UTP and signalling via GTP binding proteins are assigned to the family of P2Y purinoceptors (Fredholm *et al*., 1994; North & Barnard, 1997), these results lead to the conclusion that one or more subtypes of P2Y receptors cause inhibition of M-type K^+ channels in rat sympathetic neurons.

Uridine nucleotides activate at least four different types of P2Y receptors, namely $P2Y_2$, $P2Y_3$, $P2Y_4$, and $P2Y_6$ (North & Barnard, 1997). Previously, UTP had been found to inhibit I_M in bullfrog sympathetic neurons (at $\geqslant 50$ μM; Adams *et al*., 1982) and in glioma neuroblastoma hybrid cells (Filippov *et al*., 1994). In the latter cell type, the effect was mediated by a receptor equipotently activated by UTP and ATP. Hence, that receptor can be designated as a P_{2U} receptor (Fredholm *et al*., 1994) which pharmacologically resembles the cloned $P2Y_2$ subtypes (Lustig *et al*., 1993; Parr *et al*., 1994). In the present study, UDP and UTP were equipotent agonists and caused half maximal inhibition of I_M at about 1 μM, but ATP did not modulate I_M. Therefore, this receptor is different from the $P2Y_2$ subtype.

P2Y receptor subtypes that are preferentially activated by uridine over adenine nucleotides include the $P2Y_3$, $P2Y_4$, and $P2Y_6$ subtypes. At $P2Y_4$ receptors, UTP is more potent than UDP (Communi *et al*., 1995; Nguyen *et al*., 1995; Nicholas *et al*., 1996), whereas at the $P2Y_3$ and $P2Y_6$ subtypes, UDP is more potent than UTP (Chang *et al*., 1995; Communi *et al*.,

1996b; Webb *et al*., 1996; Nicholas *et al*., 1996). Moreover, $P2Y_4$ receptors are insensitive to ADP (Communi *et al*., 1995; Nguyen *et al*., 1995), while $P2Y_6$ receptors are insensitive to ATP (Chang *et al*., 1995; Communi *et al*., 1996b; Nicholas *et al*., 1996). Similarly, at $P2Y_3$ receptors, ADP is more potent an agonist than ATP (Webb *et al*., 1996). Hence, the finding that ADP was an agonist, although weak (half maximal inhibition at 29 μM), while ATP was inactive, suggests that $P2Y_3$- or $P2Y_6$-like receptors mediated the inhibition of I_M.

One may discriminate between the uridine nucleotide-preferring $P2Y_4$ and $P2Y_6$ receptor subtypes by use of the antagonists reactive blue 2, PPADS, and suramin: the $P2Y_4$ receptor is insensitive to suramin, but blocked by reactive blue 2 and PPADS, with PPADS being more potent than reactive blue 2 (Charlton *et al*., 1996; Communi *et al*., 1996a). In contrast, at $P2Y_6$ receptors reactive blue 2 is more potent an antagonist than PPADS (Robaye *et al*., 1997). Both $P2Y_3$ and $P2Y_6$ receptors are insensitive to suramin at concentrations <100 μM (Webb *et al*., 1996; Robaye *et al*., 1997). Thus, the order of antagonist activities reactive blue 2 > PPADS > suramin may again be taken as an indication to $P2Y_6$-like receptors mediating the effects of UTP on I_M in rat superior cervical ganglion neurons. Interestingly, none of the above antagonists attenuated UTP-induced depolarizations in whole superior cervical ganglia of adult rats (Connolly *et al*., 1993; Connolly & Harrison, 1994; Connolly, 1995). Hence, these ganglia possibly express several different subtypes of uridine nucleotide-sensitive receptors during development.

In summary, the present results show that uridine nucleotide preferring P2Y receptors of rat superior cervical ganglion neurons cause a selective inhibition of K_M channels. These receptors display a rank order of agonist potency of UTP = UDP > ADP with ATP being inactive and couple to K_M channels via GTP binding proteins.

The author wishes to thank Drs M. Freissmuth and S. Huck for critical reading of the manuscript and E. Stocker for help and advice with initial perforated-patch experiments. The perfect technical assistance by A. Motejlek is gratefully acknowledged.

References

ADAMS, P.R., BROWN, D.A. & CONSTANTI, A. (1982). Pharmacological inhibition of the M-current. *J. Physiol.,* **332,** 223 – 262.

BERNHEIM, L., MATHIE, A. & HILLE, B. (1992). Characterization of muscarinic receptor subtypes inhibiting Ca^{2+} current and M current in rat sympathetic neurons. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 9544 – 9548.

BOEHM, S. (1994). Noradrenaline release from rat sympathetic neurons evoked by P_2-purinoceptor activation. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **350,** 454 – 458.

BOEHM, S. & BETZ, H. (1997). Somatostatin inhibits excitatory transmission at rat hippocampal synapses via presynaptic receptors. *J. Neurosci.,* **17,** 4066 – 4075.

BOEHM, S., HUCK, S. & ILLES, P. (1995). UTP- and ATP-triggered transmitter release from rat sympathetic neurons via separate receptors. *Br. J. Pharmacol.,* **116,** 2341 – 2343.

BOEHM, S. & HUCK, S. (1995). α_2-Adrenoceptor-mediated inhibition of acetylcholine-induced noradrenaline release from rat sympathetic neurons: an action at voltage-gated Ca^{2+} channels. *Neuroscience,* **69,** 221 – 231.

BOEHM, S. & HUCK, S. (1997a). Receptors controlling transmitter release from sympathetic neurons *in vitro*. *Prog. Neurobiol.,* **51,** 225 – 242.

BOEHM, S. & HUCK, S. (1997b). Noradrenaline release from rat sympathetic neurons triggered by activation of B_2 bradykinin receptors. *Br. J. Pharmacol.,* **122,** 455 – 462.

BROWN, D.A., MARRION, N.V. & SMART, T.G. (1989). On the transduction mechanism for muscarine-induced inhibition of M-current in cultured rat sympathetic neurones. *J. Physiol.,* **413,** 469 – 488.

CAULFIELD, M.P., JONES, S., VALLIS, Y., BUCKLEY, N.J., KIM, G.-D., MILLIGAN, G. & BROWN, D.A. (1994). Muscarinic M-current inhibition via $G_{\alpha q/11}$ and α-adrenoceptor inhibition of Ca^{2+} currents via $G_{\alpha o}$ in rat sympathetic neurones. *J. Physiol.,* **477,** 415 – 422.

CHANG, K., HANAOKA, K., KUMADA, M. & TAKUWA, Y. (1995). Molecular cloning and functional analysis of a novel P2 nucleotide receptor. *J. Biol. Chem.,* **270,** 26152 – 26158.

CHARLTON, S.J., BROWN, C.A., WEISMAN, G.A., TURNER, J.T., ERB, L. & BOARDER, M.R. (1996). Cloned and transfected $P2Y_4$ receptors: characterization of a suramin and PPADS insensitive response to UTP. *Br. J. Pharmacol.,* **119,** 1301 – 1303.

COMMUNI, D. & BOEYNAEMS, J.M. (1997). Receptors responsive to extracellular pyrimidine nucleotides. *Trends Pharmacol. Sci.,* **18,** 83 – 86.

COMMUNI, D., MOTTE, S., BOEYNAEMS, J.M. & PIROTTON, S. (1996a). Pharmacological characterization of the human $P2Y_4$ receptor. *Eur. J. Pharmacol.,* **317,** 383 – 389.

COMMUNI, D., PARMENTIER, M. & BOEYNAEMS, J.M. (1996b). Cloning, functional expression and tissue distribution of the human $P2Y_6$ receptor. *Biochem. Biophys. Res. Comm.,* **222,** 303 – 308.

COMMUNI, D., PIROTTON, S., PARMENTIER, M. & BOEYNAEMS, J.M. (1995). Cloning and functional expression of a human uridine nucleotide receptor. *J. Biol. Chem.,* **270,** 30849 – 30852.

CONNOLLY, G.P. (1995). Differentiation by pyridoxal 5-phosphate, PPADS, and Iso-PPADS between responses mediated by UTP and those evoked by α,β-methylene-ATP on rat sympathetic ganglia. *Br. J. Pharmacol.,* **114,** 727 – 731.

CONNOLLY, G.P. & HARRISON, P.J. (1994). Reactive blue 2 discriminates between responses mediated by UTP and those evoked by ATP or α,β-methylene-ATP on rat sympathetic ganglia. *Eur. J. Pharmacol.,* **259,** 95 – 99.

CONNOLLY, G.P., HARRISON, P.J. & STONE, T.W. (1993). Action of purine and pyrimidine nucleotides on the rat superior cervical ganglion. *Br. J. Pharmacol.,* **110,** 1297 – 1304.

CONSTANTI, A. & BROWN, D.A. (1981). M-currents in voltage-clamped mammalian sympathetic neurones. *Neurosci. Lett.,* **24,** 289 – 294.

CURRIE, K.P.M. & FOX, A.P. (1996). ATP serves as a negative feedback inhibitor of voltage-gated Ca^{2+} channel currents in cultured bovine adrenal chromaffin cells. *Neuron,* **16,** 1027 – 1036.

DELEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *American Journal of Physiology,* **235,** 97 – 102.

FILIPPOV, A.K., SELYANKO, A.A., ROBBINS, J. & BROWN, D.A. (1994). Activation of nucleotide receptors inhibits M-type K current [IK(M)] in neuroblastoma × glioma hybrid cells. *Pflügers Arch.,* **429,** 223 – 230.

FILIPPOV, A.K. & BROWN, D.A. (1996). Activation of nucleotide receptors inhibits high-threshold calcium currents in NG108-15 neuronal hybrid cells. *Eur. J. Neurosci.,* **8,** 1149 – 1155.

FILIPPOV, A.K., WEBB, T.E., BARNARD, E.A. & BROWN, D.A. (1997). Inhibition by heterologously expressed $P2Y_2$ nucleotide receptors of N-type calcium current in rat sympathetic neurons. *Br. J. Pharmacol.,* **121,** 849 – 851.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.,* **46,** 143 – 156.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.,* **391,** 85 – 100.

HARDEN, T.K., BOYER, J.L. & NICHOLAS, R.A. (1995). P2-purinergic receptors: subtype-associated signaling responses and structure. *Ann. Rev. Pharmacol. Toxicol.,* **35,** 541 – 579.

HILLE, B. (1994). Modulation of ion channel function by G protein-coupled receptors. *Trends Neurosci.,* **17,** 531 – 536.

IKEUCHI, Y. & NISHIZAKI, T. (1996). P2 purinoceptor-operated potassium channel in rat cerebellar neurons. *Biochem. Biophys. Res. Comm.,* **218,** 67 – 71.

JONES, S.W. & ADAMS, P.R. (1987). The M-current and other potassium currents of vertebrate neurons In *Neuromodulation. The biochemical control of neuronal excitability*. (Kaczmarek, L.K. and Levitan, I.B. eds.), pp. 159–186, Oxford University Press.

JONES, S., BROWN, D.A., MILLIGAN, G., WILLER, E., BUCKLEY, N.J. & CAULFIELD, M.P. (1995). Bradykinin excites rat sympathetic neurons by inhibition of M current through a mechanism involving B_2 receptors and $G_{\alpha q/11}$. *Neuron,* **14,** 399–406.

LAMAS, A., SELYANKO, A.A. & BROWN, D.A. (1997). Effects of cognition-enhancer, linopirdine (DuP 996), on M-type potassium currents (IK(M)) and some other voltage- and ligand-gated membrane currents in rat sympathetic neurons. *Eur. J. Neurosci.,* **9,** 605–616.

LUSTIG, K.D., SHIAU, A.K., BRAKE, A.J. & JULIUS, D. (1993). Expression cloning of an ATP receptor from mouse neuroblastoma cells. *Proc. Natl. Acad. Sci.,* **90,** 5110–5117.

MARRION, N.V., SMART, T.G., MARSH, S.J. & BROWN, D.A. (1989). Muscarinic suppression of the M-current in the rat sympathetic ganglion is mediated by receptors of the M_1-subtype. *Br. J. Pharmacol.,* **98,** 557–573.

MARSH, S.J. & BROWN, D.A. (1991). Potassium currents contributing to action potential repolarization in dissociated cultured rat superior cervical sympathetic neurones. *Neurosci. Lett.,* **133,** 298–302.

NGUYEN, T., ERB, L., WEISMAN, G.A., MARCHESE, A., HENG, H.H.Q., GARRAD, R.C., GEORGE, S.R., TURNER, J.T. & O'DOWD, B.F. (1995). Cloning, expression and chromosomal localization of the human uridine nucleotide receptor gene. *J. Biol. Chem.,* **270,** 30845–30848.

NICHOLAS, R.A., WATT, W.C., LAZAROWSKI, E.R., LI, Q. & HARDEN, T.K. (1996). Uridine nucleotide selectivity of three phospholipase C-activating P2 receptors: identification of a UDP-selective, a UTP-selective, and an ATP- and UTP-specific receptor. *Mol. Pharmacol.,* **50,** 224–229.

NORTH, R.A. & BARNARD, E.A. (1997). Nucleotide receptors. *Cur. Op. Neurobiol.,* **7,** 346–357.

PARR, C.E., SULLIVAN, D.M., PARADISO, A.M., LAZAROWSKI, E.R., BURCH, L.H., OLSEN, J.C., ERB, L., WEISMAN, G.A., BOUCHER, R.C. & TURNER, J.T. (1994). Cloning and expression of a human P_{2U} nucleotide receptor, a target for cystic fibrosis pharmacotherapy. *Proc. Natl. Acad. Sci. U.S.A.,* **91,** 3275–3279.

PENNEFATHER, P., LANCASTER, B., ADAMS, P.R. & NICOLL, R.A. (1985). Two distinct Ca-dependent K^+ currents in bullfrog sympathetic ganglion cells. *Proc. Natl. Acad. Sci. U.S.A.,* **82,** 3040–3044.

RAE, J., COOPER, K., GATES, P. & WATSKY, M. (1991). Low access resistance perforated patch recordings using amphotericin B. *J. Neurosci. Meth.,* **37,** 15–26.

ROBAYE, B., BOEYNAEMS, J.M. & COMMUNI, D. (1997). Slow desensitization of the human P2Y6 receptor. *Eur. J. Pharmacol.,* **329,** 231–236.

SCHOFIELD, G.G. & IKEDA, S.R. (1989). Potassium currents of acutely isolated adult rat superior cervical ganglion neurons. *Brain Res.,* **485,** 205–214.

VON KÜGELGEN, I., NÖRENBERG, W., ILLES, P., SCHOBERT, A. & STARKE, K. (1997). Differences in the mode of stimulation of cultured rat sympathetic neurons between ATP and UTP. *Neuroscience,* **78,** 935–941.

WEBB, T.E., HENDERSON, D., KING, B.F., WANG, S., SIMON, J., BATESON, A.N., BURNSTOCK, G. & BARNARD, E.A. (1996). A novel G protein-coupled P2 purinoceptor (P2Y3) activated preferentially by nucleoside diphosphates. *Mol. Pharmacol.,* **50,** 258–265.

(Received January 30, 1998
Revised April 9, 1998
Accepted April 22, 1998